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| **Applicant**<br>LEE, Taekyu et al | |

1. The designated Office is hereby notified of its election made:

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   07 July 1999 (07.07.99)

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# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/07059

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K5/02

## II. FIELDS SEARCHED

Minimum Documentation Searched[7]

| Classification System | Classification Symbols |
|---|---|
| Int.Cl. 5 | C07K |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

| Category ° | Citation of Document,[11] with indication, where appropriate, of the relevant passages[12] | Relevant to Claim No.[13] |
|---|---|---|
| P,X | EP,A,361341 (MOLECULAR THERAPEUTICS) 04 April 1990 see the whole document --- | 1-6 |
| P,X | EP,A,346847 (HOFFMANN-LA ROCHE) 20 December 1989 see claim 1 --- | 1-7 |

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
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| 11 MARCH 1991 | 25. 03. 91 |
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| EUROPEAN PATENT OFFICE | M. PEIS M. Peis |

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# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9007059
SA 42756

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| EP-A-361341 | 04-04-90 | AU-A- | 4230889 | 16-08-90 |
| | | JP-A- | 2191243 | 27-07-90 |
| EP-A-346847 | 20-12-89 | AU-A- | 3613089 | 14-12-89 |
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(54) Title: PEPTIDE INHIBITORS OF HIV PROTEASE

(57) Abstract

The invention provides HIV protease inhibitors which are potential drugs for HIV infected patients or cells. Preferably, peptides are provided that have an amino alcohol core that mimics a Phe-Pro and/or Leu-Pro binding site for the protease. The core is preferably ringed by at least three amino acids. In one form, an N-terminal cap is positioned adjacent to an Asn residue.

11-2-2000

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# PEPTIDE INHIBITORS OF HIV PROTEASE

This invention was made with U.S. government support awarded by the National Institutes Of Health (NIH) Grant Nos: GM40092, DK20100, AI27302 The U.S. government has certain rights in this invention.

## Technical Field

This invention relates to uses of a modified peptide as an inhibitor of HIV protease. More particularly, it relates to incorporating amino-alcohol variants of the protease's preferred binding site in a special peptide backbone to preferentially bind and occupy the protease.

## Background Art

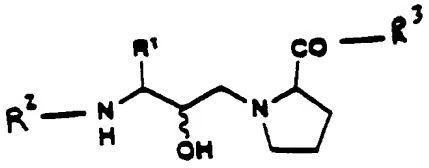
An important problem facing medical science is the development of drugs for those already infected by HIV. Inhibition of viral proteases as an approach to antiviral therapy has been demonstrated with another virus by Korant et al., 32 J. Cell. Biochem. 91-95 (1986), who showed that an inhibitor of cysteine proteases, cystatin, inhibited replication of poliovirus in tissue culture. Also, Oroszlan, Abstract AIDS Symposium (June 1987) demonstrated inhibition by p pstatin of processing of gag-pol poly

protein by purified HIV protease in vitro and in tissue culture in the presence of DMSO. The disclosure of the above articles and of all other articles referred to herein are incorporated by reference as if fully set forth herein.

The goals of any drug to be used in humans are that the drug should have high potency, high specificity, few side effects, stability, and reasonable costs of manufacture. Unfortunately, to date no drug for AIDS inhibition treatment has satisfactorily met all of these criteria.

## Disclosure Of The Invention

We identify herein peptide variants which preferentially bind to HIV protease so as to inhibit its activity. In one embodiment, the present invention provides a peptide having the moiety:




where $R_1$ is selected from the group consisting of isobutyl, benzyl, cyclohexylmethyl, and other arylalkyl or alkyl moieties with less than twelve carbons; and where $R_2$ and $R_3$ each has at least one amino acid residue, and at least one of $R_2$ or $R_3$ has at least two amino acid residues.

In another aspect, $R_2$ is also linked to the following

moiety:

Preferably, $R_2$ and $R_3$ are residues of one or more of the twenty amino acids commonly found in proteins or one of the other naturally occurring amino acids. See A. Lenhniger, Biochemistry, p.69-72 (1970). In an especially preferred form, $R_2$ contains an asparagine residue. Also, $R_1$ is preferably a side chain found on a naturally occurring amino acid.

The objects of the invention therefore include providing compositions of the above kind that are relatively inexpensive to produce and will inhibit HIV protease. These and still other objects and advantages of the present invention will be apparent from the description which follows. The following embodiments do not represent the full scope of the invention. Rather, the invention may be employed in other embodiments. Reference is therefore to be made to the claims herein for interpreting the scope of the present invention.

## Brief Description Of The Drawings

Fig. 1 depicts the synthesis of half of a peptide of the leucine amino alcohol type;

Fig. 2 depicts the synthesis of the other half of the molecule;

Fig. 3 depicts the synthesis of three final products;

Fig. 4 is similar to Fig. 1, but depicts phenyl variants;

Fig. 5 is similar to Fig. 3, but d picts phenyl variants;

Fig. 6 depicts another system for protecting one end of th final product;

Fig. 7 depicts the synthesis of one-half of another peptide;

Fig. 8 depicts the sythesis of two variants of the other half of the peptide;

Fig. 9 depicts two coupling reactions; and

Fig. 10 depicts two N-terminal caps.

## Best Modes For Carrying Out The Invention

### General Procedures

In the discussion below, the following abbreviations are used: AcOH - acetic acid; Boc - t-butoxycarbonyl; BuOH - butanol; DCM - dichloromethane; DMF - N,N-dimethylformamide; DMSO - dimethylsulphoxide; EDCI - N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide; HOBt - 1-hydroxybenzotriazole; MeOH - methanol; NMM - N-methylmorpholine; Z - benzyloxycarbonyl.

A: Isobutyl chloroformate mediated coupling reactions:

To a cold (-25°C) solution of Nα-protected amino acid (100 mmol) in dry dichloromethane (200-400 mL) is added N-methylmorpholine (100 mmol) followed by careful addition of isobutyl chloroformate (100 mmol) maintaining the temperature of the solution between -15 and -25°C. The solution is stirred at -25°C for a further 20 minutes, then a solution of amino acid or peptide ester hydrohalide or toluenesulphonate salt (100 mmol) in DMF and/or DCM is added followed by addition of NMM (100 mmol). The reaction is stirred for 1 hour at -20°C and for a further 1-3 hours at room temperature. The solvent is removed in vacuo and the residue taken up in ethyl acetate and washed

with saturated $NaHCO_3$ (x2), 5% citric acid solution, saturated $NaHCO_3$, water and brine, and dried over $MgSO_4$. Evaporation of the solvent yields a solid which is recrystallized from a suitable solvent combination.

B: EDCI/HOBt mediated peptide couplings:

Boc amino acid and HOBt (1.5 equivalent) are dissolved in dry DMF (2-5 mL per mmol) and cooled to 0°C. EDCI (1.1 equivalent) is added and the solution stirred for 30-40 minutes. A solution of amino acid or peptide, ester or chloromethylketone, amine hydrochloride (0.9 equivalent) in DMF (1-5 mL per mmol) is added, followed by NMM (0.9 equivalent). The reaction is stirred for 1-2 hours at 0°C and overnight at room temperature. The reaction is worked up by direct precipitation of the crude product from a concentrated DMF solution by careful addition of a large volume of 60% saturated $NaHCO_3$. The precipitate is collected by filtration over a hardened filter paper (Whatman No. 50 or 54), and the solid washed thoroughly with saturated $NaHCO_3$ (200-500 mL), water (200-500 mL), 5% citric acid solution (200-500 ml), and water (200-500 mL). The product is dried in vacuo, redissolved in DMF, filtered through a glass wool plug and reprecipitated by addition of water. Again the product is filtered, washed with water, and dried.

C: Cleavage of Boc protecting group with 4N HCl in dioxane:

The Boc-protected peptide or amino acid derivative is dissolved in 4N HCl in dioxane (Pierce) (6-40 equivalents) and stirred at room temperature for 30-60 minutes, the course of reaction being monitored by thin layer chromatography. Once complete, the solution is evaporated to dryness at <30°C and the residue evaporated from anhydrous ether (x3) to remove traces of HCl. The crude product is dried in vacuo in a desiccator over NaOH pell ts overnight and is used without further purification or characterization.

D: Preparation of amino acid diazomethyl ketones:

Nα-Protected amino acid (50 mmol) is dissolved in dry THF (100 mL) and cooled to -25°C. NMM (50 mmol) is added, followed by isobutylchloroformate (50 mmol), with the temperature being maintained at -25°C during addition. After stirring for 5 minutes anhydrous ether (100 mL) is added while simultaneously cooling the solution to -70°C. The cold solution is quickly filtered under a blanket of $N_2$ and the cold filtrate treated with a solution containing approximately 70 mmol of diazomethane in ether (250 ml). The reaction is allowed to gradually warm up to room temperature and excess diazomethane is removed by purging the solution with a stream of $N_2$ for 15-30 minutes. The solution is evaporated to dryness and the residue taken up in ether (200 mL), and washed with saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. The product may be crystallized from ether-hexane or hexane.

E: Conversion of diazomethylketones to halomethylketones:

The amino acid diazomethylketone is dissolved in anhydrous dioxane. After slow addition of the first equivalent of HCl in dioxane is complete (slow addition is necessitated by the rapid evolution of nitrogen), a further 6-10 equivalents is added and the cooling bath removed. The reaction is stirred at room temperature for 1 hour and the product precipitated by addition of ether. The product is filtered, washed with copious amounts of ether, and dried *in vacuo* over NaOH pellets. Prolonged storage of these compounds results in significant colouration and decomposition.

F: Acetylation of peptides:

A solution of the peptide amine hydrochloride in dry DMF (10-20 mL/g) is cooled to 0°C in an ice-water bath and neutralized with triethylamine (2.1-2.5 equivalents). A solution of acetyl chloride in DCM (0.3-1.8 M) is then added dropwise and the reaction left to stir for 1 hour at

0°C, and for a further 1 hour at room temperature. The solution is then concentrated to a small volume under high vacuum, and water is added to precipitate the acetylated peptide, which is collected by filtration, and washed with water, before drying in vacuo over NaOH pellets.

G: Preparation of Aminoketones:

Nα-Protected peptide chloromethylketone derivatives (1.0 mmol) are dissolved in DMF (4-7 mL per mmol) and NaI (1.1 mmol) added and the mixture stirred for 15 minutes. The peptide ester toluenesulphonate salt (1.1 mmol) in DMF (2-5 mL per mmol) is added followed by $NaHCO_3$ (2.1 mmol) and the reaction left to stir for 12-18 hours at room temperature. Upon complete reaction, the solution is diluted with ethyl acetate (50-70 ml), and washed with water (1N $KHSO_4$ may also be used, though the potential solubility of an amine-containing peptide must be considered). The aqueous layer is extracted with ethyl acetate (x2) and the combined extracts washed with water and brine before drying over $Na_2SO_4$. The product thus obtained is of good purity, though silica gel chromatography carried out to remove trace impurities contributes to epimerisation at the chiral centre α to the ketone carbonyl.

H. Reduction of aminoketones to aminoalcohols:

The crude aminoketone (1.0 mmol) is taken up in anhydrous MeOH (10 mL), cooled to 0°C and treated with $NaBH_4$ (2.5 equiv.) in a single portion. The course of reaction is monitored by thin layer chromatography, and addition of extra $NaBH_4$ is occasionally necessary. Upon completion of reduction, the solution is treated with ethyl acetate (50 mL) and water (50 mL). The aqueous phase is extracted with ethyl acetate (x2) and the combined extracts washed with water and brine, and dried over $Na_2SO_4$. The product is purified by silica gel chromatography using a gradient of methanol in chloroform

for optimal separations.

I. Hydrogenolytic deprotection of final products:

The amino alcohol containing peptides (50 µmol) are dissolved in 90% acetic acid (3.5 mL) and 20% palladium hydroxide on charcoal (Pearlman's catalyst) is added under an $N_2$ atmosphere. Deprotection is achieved by passage of $H_2$ as a steady stream through the solution for 3-4 hours. The catalyst is removed by filtration through pre-washed and swollen Celite, and the filtrate concentrated in vacuo to approximately 1 mL total volume. The solution is diluted with water (20 ml), washed with chloroform (x3), and re-concentrated to 5-8 mL total volume. The solution is filtered through a glass wool plug, and lyophilized to yield the final product peptide as a fine colourless powder.

Examples

1.1 (3S)-t-Butoxycarbonylamino-1-diazo-5-methyl-2-hexanone, (BocLeuCHN$_2$). Compound 1.1 was prepared according to general procedure D from Boc leucine monohydrate (13.89 g, 55.7 mmol). The product was crystallized from ether-hexane.

1.2 (3S)-Amino-1-chloro-5-methyl-2-hexanone hydrochloride, (Cl-H$_2$+LeuCH$_2$Cl). Compound 1.2 was prepared from Compound 1.1 (5.46 g, 21.4 mmol) in ether (21 mL), and a solution of 4N HCl in dioxane (25 mL, 100 mmol) as described in general procedure E.

1.3 (3S)-(N-t-Butoxycarbonylasparaginyl)amino-1-chloro-5-methyl-2-hexanone, (BocAsnLeuCH$_2$Cl). Compound 1.3 was prepared from Boc asparagine (3.45 g, 14.9 mmol) and Compound 1.2 (2.70 g, 13.5 mmol) according to general procedure B.

1.4 (3S)-(N-t-butoxycarbonylleucinylasparaginyl)amino-1-chloro-5-methyl-2-hexanon , (BocLeuAsnLeu$CH_2Cl$). Compound 1.4 was prepared by deprotection of Compound 1.3 (5.78 g, 15.3 mmol) according to general procedure C, and coupling with Boc leucine monohydrate (4.16 g, 16.7 mmol) according to general procedure B.

1.5 (3S)-N-Acetylleucinylasparaginyl)amino-1-chloro-5-methyl-2-hexanone, (AcLeuAsnLeu$CH_2Cl$). Compound 1.5 was prepared by deprotection of Compound 1.4 (0.99 g, 2.02 mmol) according to general procedure C, followed by acetylation by general procedure F.

1.6 (3S)-N-t-Butoxycarbonyl-O-benzylserinylleucinyl-asparaginyl)-amino-1-chloro-5-methyl-2-hexanone, (BocSer(Bzl)LeuAsnLeu$CH_2Cl$). Compound 1.6 was prepared by deprotection of Compound 1.4 (1.14 g, 2.32 mmol) according to general procedure C, and coupling with Boc serine benzyl ether (0.77 g, 2.61 mmol) according to general procedure B.

1.7 (3S)-N-Acetyl-O-benzylserinylleucinylasparaginyl)-amino-1-chloro-5-methyl-2-hexanone, (AcSer(Bzl)LeuAsnLeu$CH_2Cl$). Compound 1.7 was prepared by deprotection of Compound 1.6 (643 mg, 0.96 mmol) according to general procedure C, followed by acetylation according to general procedure F.

2.1 Nα-t-Butoxycarbonylisoleucylvaline methyl ester, (BocIleValOMe). Compound 2.1 was prepared from valine methyl ester hydrochloride (19.99 g, 119 mmol) and Boc isoleucine hemihydrate (26.05 g, 108 mmol) according to general procedure A, and recrystallized from ethyl acetate-hexane.

2.2 N-Benzyloxycarbonylprolylisoleucylvaline methyl ester, (ZProIleValOMe). Compound 2.2 was prepared from Compound 2.1 (14.00 g, 40.6 mmol) by deprotection according to general procedur C, followed by coupling with Z-prolin (10.09 g, 40.5 mmol) according to general

procedure A. The product was recrystallized from ethyl acetate-hexane.

2.3 Prolylisoleucylvaline methyl ester tosylate, (TosO-$H_2$+ProIleValOMe). Compound 2.3 (5.11 g, 10.7 mmol) was deprotected by catalytic hydrogenation as follows. The protected tripeptide was dissolved in methanol (130 mL) together with p-toluenesulphonic acid (2.17 g, 11.4 mmol), and 10% palladium on charcoal (0.5 g) was added under an $N_2$ atmosphere. Hydrogen gas was bubbled through the mixture for 16 hours. Then the solution was filtered through celite to remove the catalyst, and evaporated to dryness to reveal the tripeptide as a foam. The excess p-toluenesulphonic acid was removed by trituration with ether and the tripeptide salt stored sealed under $N_2$ as a hygroscopic glass.

3.1 (2RS,3S)-3-(N-Acetylleucinylasparaginyl)-amino-2-hydroxy-5-methyl-1-(N-prolylisoleucylvaline methyl ester)-hexane, (AcLeuAsnLeu*(CHOHCH$_2$N)ProIleValOMe). Compound 3.1 was prepared from Compound 1.5 (133 mg, 308 µmol) and Compound 2.3 (235 mg, 457 µmol) in DMF (4 mL) as described in general procedure G. The crude product was reduced without further purification using $NaBH_4$ (26 mg, 679 µmol) as described in general procedure H. The crude product was purified by chromatography on silica gel, using a gradient of 5-11% MeOH in $CHCl_3$.

3.2 (2RS,3S)-3-(N-Acetyl-O-benzylserinylleucinylasparaginyl)-amino-2-hydroxy-5-methyl-1-(N-prolylisoleucylvaline methyl ester)-hexane, (AcSer(Bzl)LeuAsnLeu*(CHOHCH$_2$N)ProIleValOMe). Compound 3.2 was prepared from Compound 1.7 (160 mg, 262 µmol) and Compound 2.3 (201 mg, 392 µmol)in DMF (4.5 mL) as described in general procedure G. The crude product was reduced without further purification as described in general procedure H, and purified by chromatography on silica gel, using a gradient of 5-11% MeOH in $CHCl_3$.

3.3 (2RS,3S)-3-(N-Acetylserinylleucinylasparaginyl)-amino-2-hydroxy-5-methyl-1-(N-prolylisoleucylvaline methyl ester)-hexane, (AcSerLeuAsnLeu* ($CHOHCH_2N$) ProIleValOMe.AcOH). Compound 3.2 was prepared from Compound 3.2 (46 mg, 50.5 μmol) by hydrogenation over palladium hydroxide on charcoal (16 mg) in 90% acetic acid as described in general procedure I.

4.1 (3S)-t-Butoxycarbonylamino-1-diazo-4-phenyl-2-butanone, (BocPheCH$N_2$). Compound 4.1 was prepared according to general procedure D from Boc phenylalanine (13.28 g, 50.0 mmol). The product was crystallized from ether-hexane.

4.2 (3S)-Amino-1-chloro-4-phenyl-2-butanone hydrochloride, (Cl-$H_2$+PheC$H_2$Cl). Compound 4.2 was prepared from Compound 4.1 (10.01 g, 34.6 mmol) in dioxane (25 mL), and a solution of 4N HCl in dioxane (50 mL, 200 mmol) as described in general procedure E.

4.3 (3S)-(N-t-Butoxycarbonylasparaginyl)amino-1-chloro-4-phenyl-2-butanone, (BocAsnPheC$H_2$Cl). Compound 4.3 was prepared from Compound 4.2 (7.04 g, 30.1 mmol) and Boc asparagine (7.66 g, 33.0 mmol) in DMF (100 mL), as described in general procedure B.

4.4 (3S)-N-t-Butoxycarbonylleucinylasparaginyl)amino-1-chloro-4-phenyl-2-butanone, (BocLeuAsnPheC$H_2$Cl). Compound 4.4 was prepared by deprotection of Compound 4.3 (9.74 g, 23.7 mmol) according to general procedure C, followed by coupling with Boc leucine (6.55 g, 26.3 mmol) (monohydrate dried together with HOBt hydrate by repeated azeotropic removal of water from DMF-toluene solution) in DMF (70 mL), as described in general procedure B.

4.5 (3S)-(N-Acetylleucinylasparaginyl)amino-1-chloro-4-phenyl-2-butanone, (AcLeuAsnPheC$H_2$Cl). Compound 4.5 was prepared by deprotection of Compound 4.4 (213 mg, 0.41 mmol) according to general procedure C, and subsequently acetylated according to g neral procedure F.

4.6 (3S)-(N-t-Butoxycarbonyl-O-benzylserinylleucinylasparaginyl)-amino-1-chloro-4-phenyl-2-butanone, (BocSer(Bzl)LeuAsnPheCH2Cl). Compound 4.6 was prepared by deprotection of Compound 4.4 according to general procedure C, followed by coupling with Boc serine benzyl ether (0.99 g, 1.89 mmol) in DMF (11 mL), as described in general procedure B.

4.7 (3S)-(N-Acetyl-O-benzylserinylleucinylasparaginyl)-amino-1-chloro-4-phenyl-2-butanone, (AcSer(Bzl)LeuAsnPhe$CH_2$Cl). Compound 4.7 was prepared from Compound 4.6 (674 mg, 0.96 mmol), by deprotection according to general procedure C, and acetylation according to general procedure F.

5.1 (2RS,3S)-3-(N-Acetylleucinylasparaginyl)-amino-2-hydroxy-4-phenyl-1-(N-prolylisoleucylvaline methyl ester)-butane, AcLeuAsnPhe*(CHOH$CH_2$N)ProIleValOMe. Compound 5.1 was prepared from Compound 4.5 (149 mg, 319 µmol) and Compound 2.3 (247 mg, 481 µmol) in DMF (4 mL) as described in general procedure G. The crude product was reduced without further purification using $NaBH_4$ (29 mg, 767 µmol) as described in general procedur H. The crude product was purified by chromatography on silica gel, using a gradient of 5-11% MeOH in $CHCl_3$.

5.2 (2RS,3S)-3-(N-Acetyl-O-benzylserinylleucinylasparaginyl)-amino-2-hydroxy-4-phenyl-1-(N-prolylisoleucylvaline methyl ester)-butane, AcSer(Bzl)LeuAsnPhe*(CHOH$CH_2$N)ProIleValOMe. Compound 5.2 was prepared from Compound 4.7 (188 mg, 292 µmol) and Compound 2.3 (223 mg, 435 µmol) in DMF (4.5 mL) as described in general procedure G. The crude product was reduced without further purification using $NaBH_4$ (28 mg, 733 µmol) as described in general procedure H. The crude product from aqueous work up was dried by azeotropic removal of water to prevent precipitation and purified by chromatography on silica gel, using a gradient of 5-11% MeOH in $CHCl_3$.

5.3 (2RS,3S)-3-(N-Acetylserinylleucinylasparaginyl)-amino-2-hydroxy-4-phenyl-1-(N-prolylisoleucylvaline methyl ester)-butane, (AcSerLeuAsnPhe*(CHOHCH$_2$N)ProIleValOMe.AcOH). Compound 5.3 was prepared from Compound 5.2 (36 mg, 37.8 µmol) by hydrogenation over palladium hydroxide on charcoal (14 mg) in 90% acetic acid as described in general procedure I.

6.1 (2RS,3S)-3(N-[2-aminobenzoyl]-leucinyl-asparaginyl)-amino-2-hydroxy-4-phenyl-1-(N-prolyl-isoleucyl-valyl methyl ester)-butane. (Abz-Leu-Asn-Phe*(CHOHCH$_2$)Pro-Ile-Val-OMe). A BOC protected variant of Compound 3.1, Compound 3.4, (102 mg) was deprotected with 4N HCl in dioxane (4 ml) as described in general procedure C, and subsequently coupled with 2-aminobenzoic acid (29 mg) using EDCl (42 mg) and HOBt (40 mg) as described in general procedure B (2-aminobenzoic acid was not pre-activated). The product was precipitated from DMF by addition of 60% saturated $NaHCO_3$. The precipitate was filtered and the solid washed thoroughly with saturated $NaHCO_3$ and water. The product was dried in vacuo, and purified by preparative thin layer chromatography on 0.5 mm plates using 10% MeOH in $CHCl_3$ as eluent.

7.1 (3S)-(N-Benzyloxycarbonylasparaginyl)amino-1-chloro-4-phenyl-2-butanone. (ZAsnPheCH$_2$Cl). Compound 7.1 was prepared from Compound 4.2 (0.78g, 3.35mmol) and Z-asparagine (0.94g, 3.53mmol) in DMF (20ml) and DCM (15ml), as described in general procedure B.

8.1 N-t-Butoxycarbonylisoleucylphenylalanine methyl ester. (BocIlePheOMe). Compound 8.1 was prepared from phenylalanine methyl ester hydrochloride (1.72g. 8.00mmol) and Boc-isoleucine hemihydrate (1.93g, 8.00mmol) according to general procedure A, and recrystallized from ethyl acetate-hexane.

8.2 N-t-Butoxycarbonylprolylisoleucylphenylalanine methyl ester. (BocProIlePheOMe). Compound 8.2 was prepared from Compound 8.1 (1.00g, 2.55mmol) by

deprotection according to general procedure C followed by coupling with Boc-proline (0.576g, 2.675mmol) as follows: Boc-proline and HOBt (0.568g, 4.20mmol) were dissolved in DCM (15ml) and cooled to 0°C. EDC1 (0.513g, 2.675mmol) was added and the solution stirred for 30 min. The deprotected Compound 8.1 in DMF (3ml) was added, followed by NMM (0.31ml, 2.80mmol). The reaction was stirred for 10-12 hours at room temperature. The reaction mixture was diluted with ethyl acetate (50ml), washed with saturated $NaHCO_3$ (15ml), 5% citric acid solution (15ml), brine (25ml) and dried over $MgSO_4$. The concentrated product was purified by silica gel chromatography with 50% ethyl acetate in hexane.

8.3 N-t-Butoxycarbonylisoleucyl-3-(2-naphthyl)alanine methyl ester (BocIle-(2-Nap)AlaOMe). Compound 8.3 was prepared from 3-(2-naphtyl)alanine methyl ester hydrochloride (0.616g, 2.32mmol) and Boc-isoleucine hemihydrate (0.614g, 2.55mmol) according to the procedure described for Compound 8.2.

8.4 N-t-Butoxycarbonylprolylisoleucyl-3-(2-naphthyl)alanine methyl ester. (BocProIle-(2-Nap)AlaOMe). Compound 8.4 was prepared from Compound 8.3 (0.75g, 1.69mmol) by deprotection according to general procedure C, followed by coupling with Boc-proline (0.39g, 1.78mmol) according to the procedure described for Compound 8.2.

9.1 (2RS, 3S)-3-(N-Benzyloxycarbonylasparaginyl)-amino-2-hydroxy-4-phenyl-1-(N-prolylisoleucylphenylalanine methyl ester)-butane. (ZAsnPhe*($CHOHCH_2N$)ProIlePheOMe). Compound 9.1 was prepared from Compound 8.2 (0.31g, 0.633mmol) by deprotection according to general procedure C, followed by the reaction with Compound 7.1 (0.254g,0.57mmol) in DMF (7ml) as described in general procedure G. The crude product was reduced without further purification using $NaBH_4$ (54mg, 1.425mmol) as described in general proc dure H. Th crude product was purified by silica g l chromatography with 3% M OH in

$CHCl_3$.

9.2 2Rs,3S)-3-(N-Benzyloxycarbonylasparaginyl)-amino-2-hydroxy-4-phenyl-1-(N-prolylisoleucyl-3-[2-naphthyl]alanine methyl ester)-butane. (ZAsnPhe*($CHOHCH_2N$)ProIle-(2-Nap)AlaOMe). Compound 9.2 was prepared from Compound 8.4 (0.28g, 0.517mmol) by deprotection according to general procedure C, followed by the reaction with Compound 7.1 (0.208g, 0.466mmol) in DMF (6ml) as described in general procedure G. The crude product was reduced without further purification using $NaBH_4$ (44mg, 1.165mmol) as described in general procedure H. The crude product was purified by silica gel chromatography with 3% MeOH in $CHCl_3$.

10.1 (2RS, 3S)-3-(N-2-Quinolylcarbonylasparaginyl)-amino-2-hydroxy-4-phenyl-1-(N-prolylisoleucylphenylalanine methyl ester)-butane. (QuAsnPhe*($CHOHCH_2N$)ProIlePheOMe). Compound 9.1 (43.6mg, 0.054mmol) was deprotected by catalytic hydrogenation as described for Compound 2.3, using palladium hydroxide on carbon (10mg) and p-toluenesulphonic acid (10.5mg, 0.054mmol), followed by coupling with 2-quinolinecarboxylic acid (10.5mg, 0.059mmol) as follows. 2-Quinolinecarboxylic acid and HOBt (11.7mg, 0.086mmol) were dissolved in DCM (4ml) and cooled to 0°C EDCI (11.5mg) was added and the solution stirred for 30 min. The deprotected Compound 9.1 in DMF (3ml) was added, followed by NMM (0.007ml, 0.06mmol). The reaction was stirred for 10-12 hours at room temperature. The reaction mixture was diluted with DCM (20ml), washed with saturated $NaHCO_3$ (5ml), water (10ml), brine (10ml) and dried over $MgSO_4$. the concentrated product was purified by silica gel chromatography with 3% MeOH in $CHCl_3$.

10.2 (2RS, 3S)-3-(N-2-Quinolylcarbonylasparaginyl)-amino-2-hydroxy-4-phenyl-1-(N-prolylisoleucly-3-[2-naphthyl]alanin methyl ester) - butane. (QuAsnPhe*($CHOHCH_2N$)ProIl -(2-Nap)AlaOMe. Compound 9.2

(140mg, 0.16mmol) was deprotected by catalytic hydrog nation as described for Compound 2.3, using palladium hydroxide on carbon (40mg) and p-toluenesulphonic acid (32mg, 0.16mmol), followed by coupling with 2-quinolinecarboxylic acid (31mg, 0.18mmol) as described for Compound 10.1. The crude product was purified by silica gel chromatography with 3% MeOH in $CHCl_3$.

Other Variants

It should be appreciated that many other peptides of this type can readily be synthesized using analogous procedures. One builds out from a core which is the ketone precursor of the amino-alcohol. The leucine variant or phenyl variant are preferred, but at $R_1$ various other naturally occurring or synthetic variant amino acid side chains (preferably low aryl or low alkyl groups) can be chosen (such as cyclohexyl methyl). Thereafter, one links a series of amino acid residues on one side of the core to build half the peptide. During these reactions, the free nitrogen can be protected. Separately, one then builds the other half of the peptide. Thereafter, one then couples the two halfs together and creates the alcohol.

Preferably, the final product has an Asn moiety in it. Also, the N-terminal end can have the nitrogen protected (e.g. with a group such as acetyl, t-butoxy-carbonyl, benzyloxycarbonyl, aminobenzoyl, benzoyl or very low alkyl (or $C_1$-$C_5$)). At the other end of the moiety, various groups such as OH or low alkyl, low aryl, esters or amides can be used.

Substitutions for hydrogen on the carbons on the proline ring and at the carbon linking the proline ring to the alcohol carbon ar also within the scope of the

inv ntion. In an especially preferred form, an N-terminal cap contains an aryl residue. However, many other higher and lower carbon containing aryl, arylalkyl, and alkyl moieties should also work as N-terminal caps. For example, the claim 7 end terminal caps could have a nitrogen substitute for a carbon on the aryl ring, and/or the hydrogens on the rings replaced by various substitutes.

It will be appreciated that the above description deals only with the preferred embodiments of the invention. A number of other modifications and changes are intended to be within the scope of the invention. The claims should therefore be looked to in judging the full scope of the invention.

## Inhibition

In addition to testing on live animals, inhibition potential can be tested by the ability to inhibit retroviral proteases with synthetic substrates and with a murine sarcoma virus Pr65gag in the assay of Katoh et al., 329 Nature 654-656 (1987). The inhibitory potency of each analog is preferably determined by using an assay analogous to that reported by J. Schneider et al. 54 Cell. 363 (1988). Briefly, synthetic HIV protease and the inhibitor are preincubated for 10 minutes in the buffer solution, and then a substrate, e.g. Ac-Thr-Ile-Met-Met-Gln-Arg-$NH_2$, is added. The reaction mixture is maintain d at 25°C. At fixed periods of time, the reaction is stopped by the addition of 10% TFA, and the formation of product monitored by analysis by HPLC. Examples of claimed compounds (final form) have been tested and shown $IC_{50}$ values of 1nM or smaller.

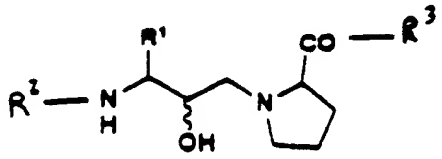
Industrial Applicability

The present invention provides a means to inhibit the ability of HIV protease to act. This should permit further understanding as to the mechanism of the enzyme and thus the virus. It also should provide potential drugs.

## Claims

We claim:

1. A peptide having the moiety:



where $R_1$ is selected from a group consisting of isobutyl, benzyl, cyclohexylmethyl, and other arylalkyl or alkyl moieties with less than twelve carbons;

where $R_2$ and $R_3$ each has at least one amino acid residue; and

where at least one of $R_2$ or $R_3$ has at least two amino acid residues.

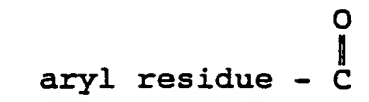
2. The peptide of claim 1, wherein $R_1$ is a side chain from a naturally occurring amino acid.

3. The peptide of claim 2, wherein $R_2$ has a residue of asparagine.

4. The peptide of claim 3, wherein opposite ends of the peptide both have protecting groups.

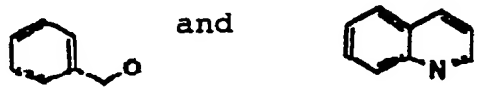
5. The peptide of claim 4, wherein a 2-aminobenzoic acid protecting moiety is one of the protecting groups.

6. The peptid of claim 1, wherein $R_2$ is also linked to a moiety:



aryl residue - C -

7. The peptide of claim 6, wherein, the aryl residue is selected from the group consisting of:



and

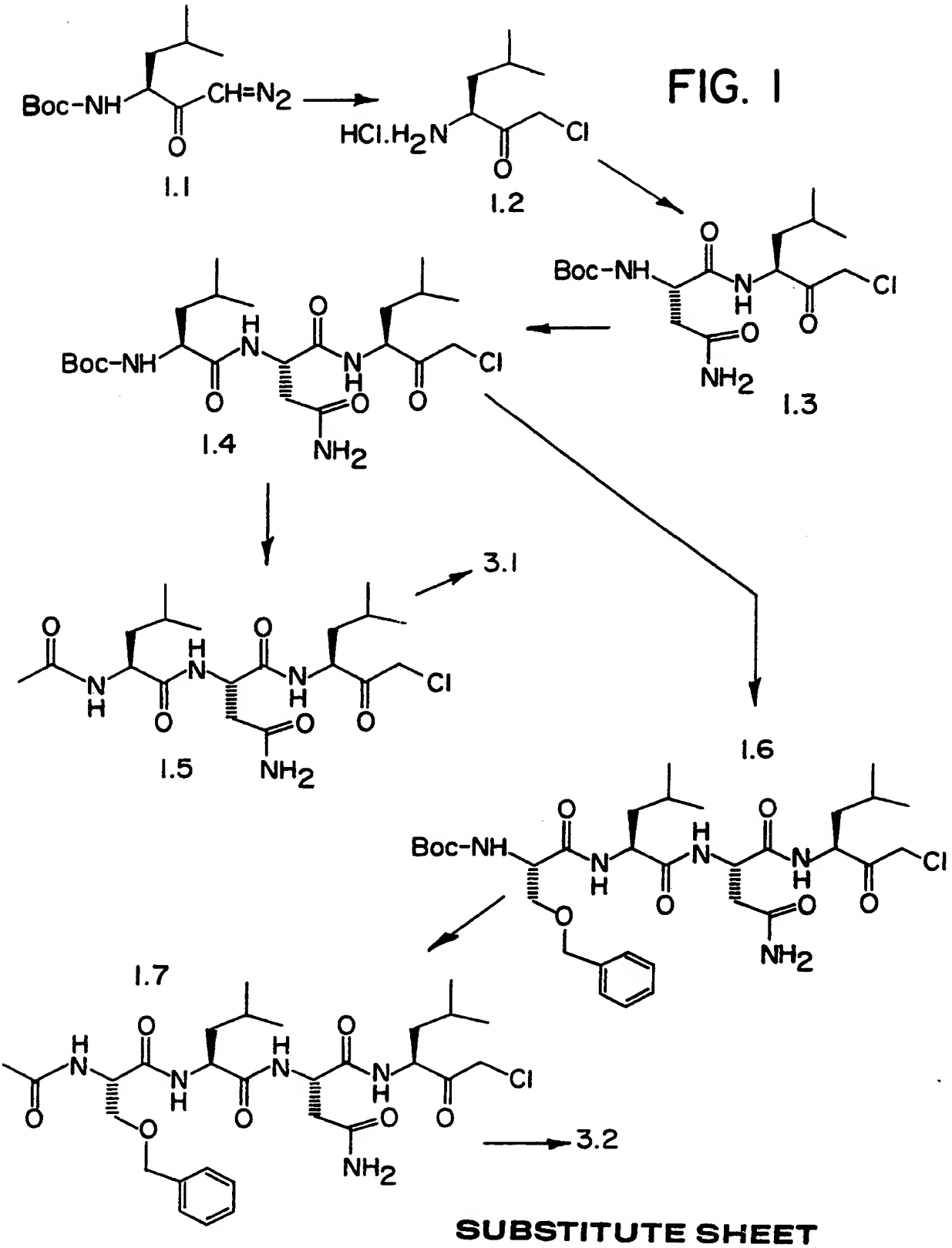

Boc-NH
CH=N2
1.1
HCl.H2N
Cl
1.2
Boc-NH
Cl
NH2
1.3
Boc-NH
Cl
NH2
1.4
3.1
Cl
NH2
1.5
1.6
Boc-NH
Cl
NH2
1.7
Cl
NH2
3.2

FIG. 1

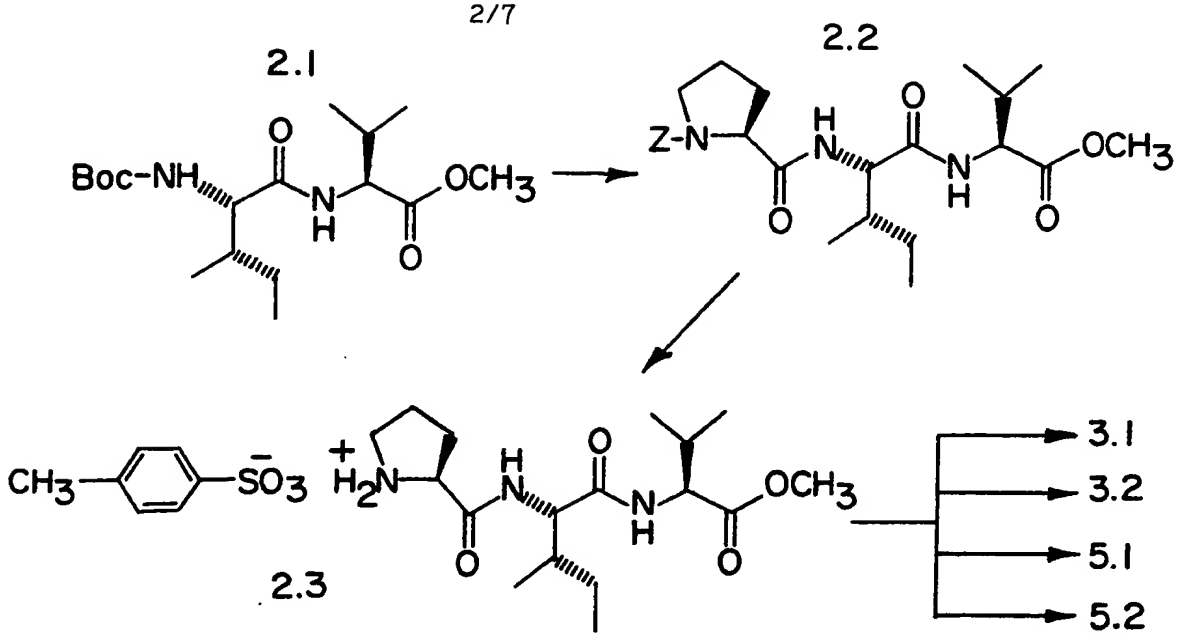

2.1
2.2
Boc-NH
OCH3
Z-N
CH3
SO3
2.3
3.1
3.2
5.1
5.2

FIG. 2

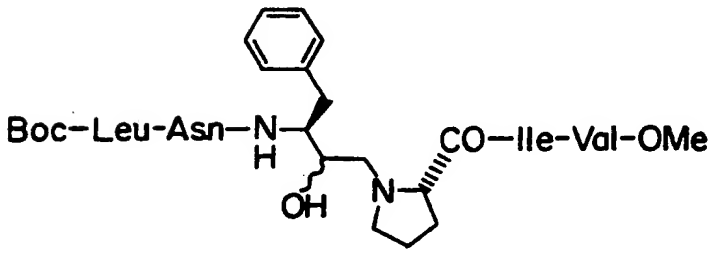
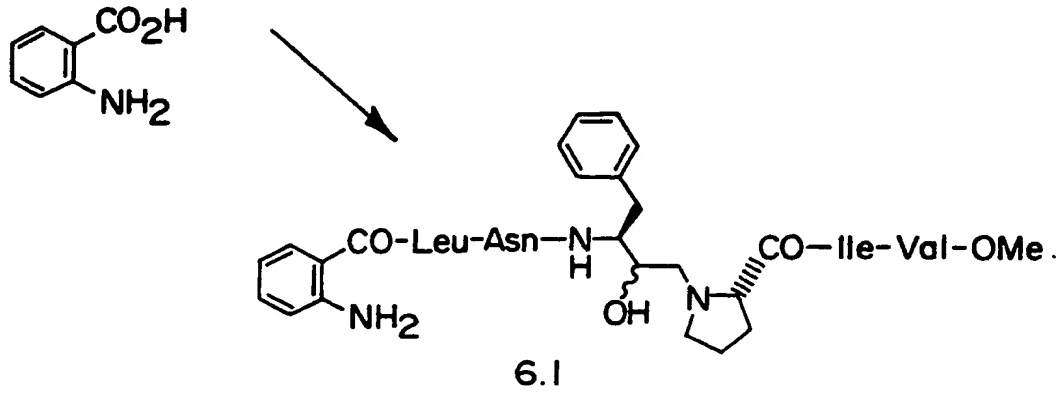

Boc-Leu-Asn-
CO-Ile-Val-OMe
OH

FIG. 6


CO2H
NH2
CO-Leu-Asn-
CO-Ile-Val-OMe
OH
6.1

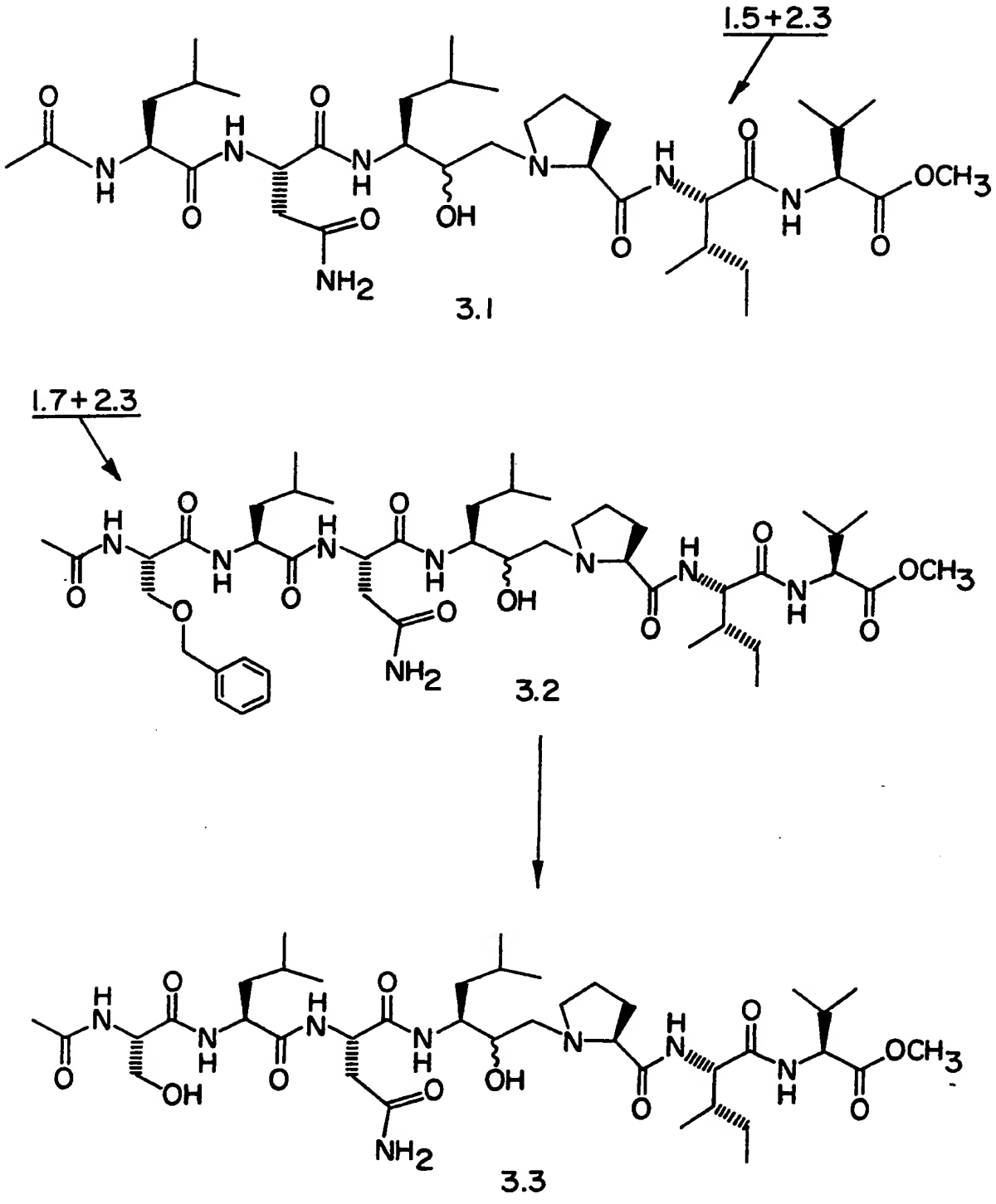
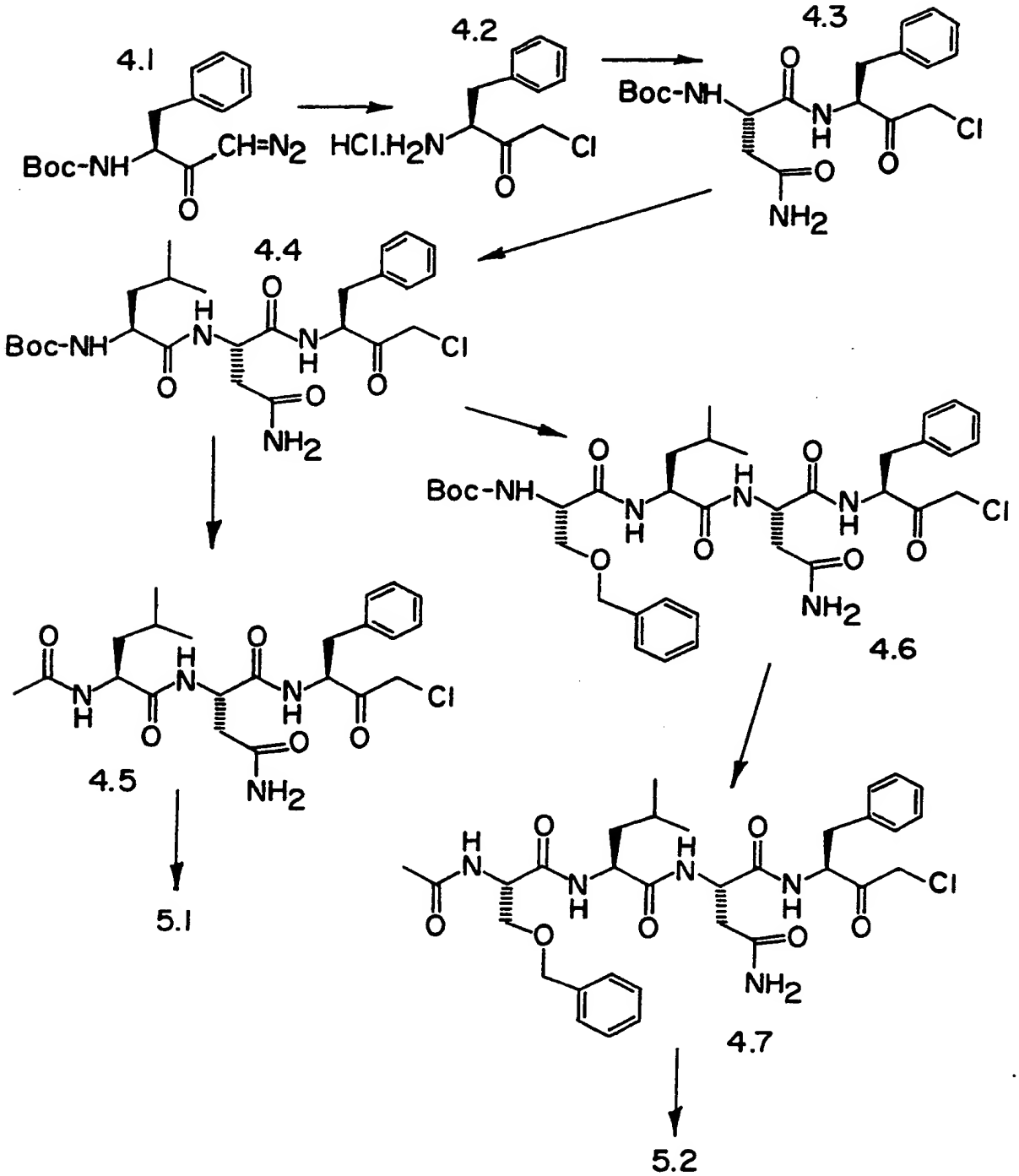
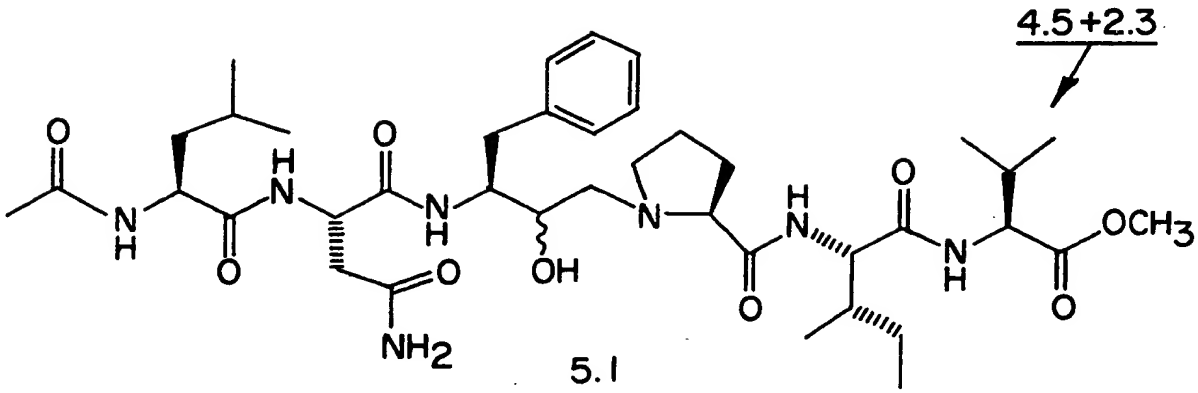



FIG. 3

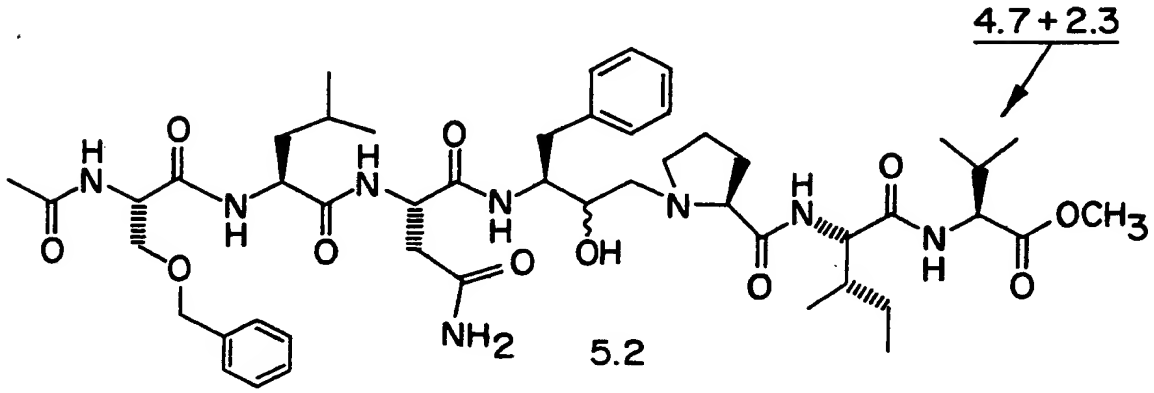
FIG. 4


4.1
Boc-NH
CH=N2
4.2
HCl.H2N
Cl
4.3
Boc-NH
Cl
NH2
4.4
Boc-NH
Cl
NH2
4.5
NH2
5.1
Boc-NH
Cl
NH2
4.6
4.7
NH2
5.2

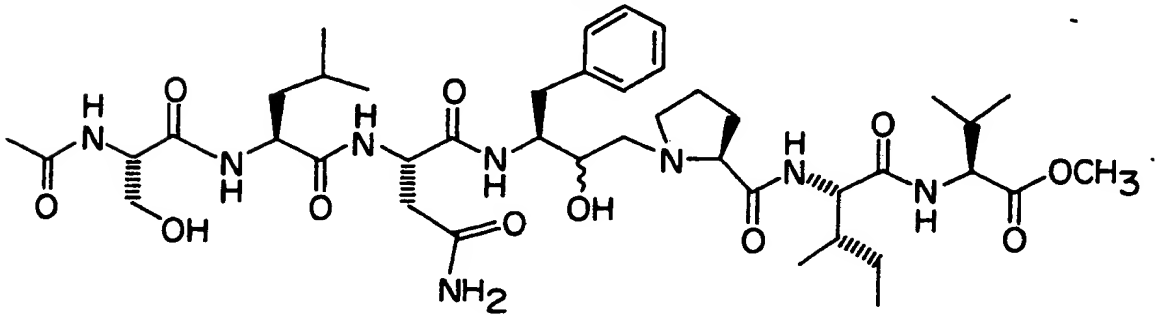



5.1

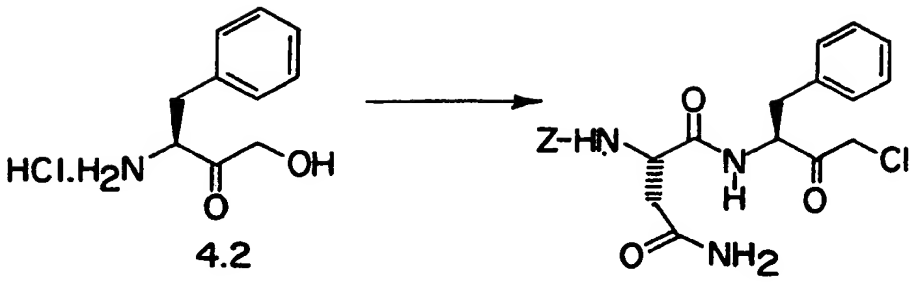
FIG. 5




5.2

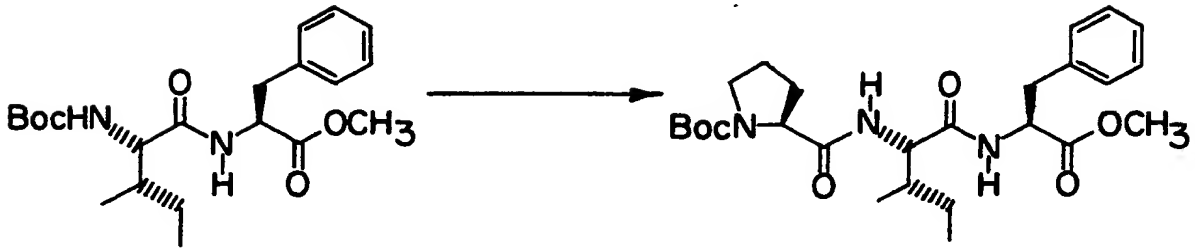



5.3

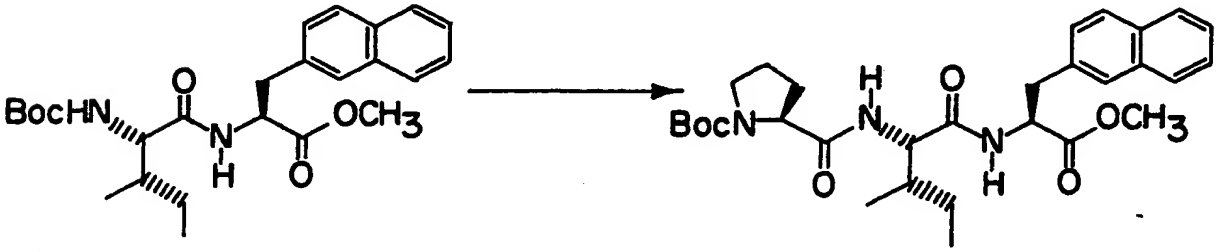



4.2 7.1

FIG. 7




8.1 8.2




8.3 8.4

FIG. 8

FIG. 9

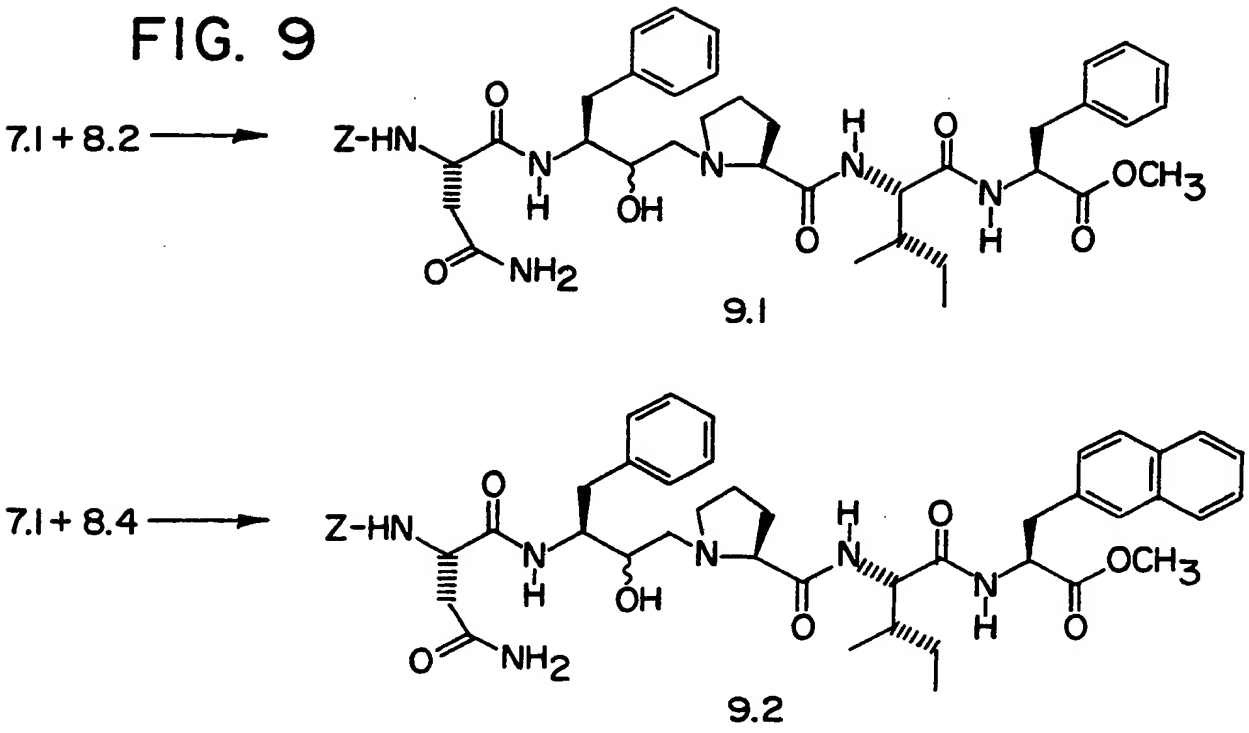
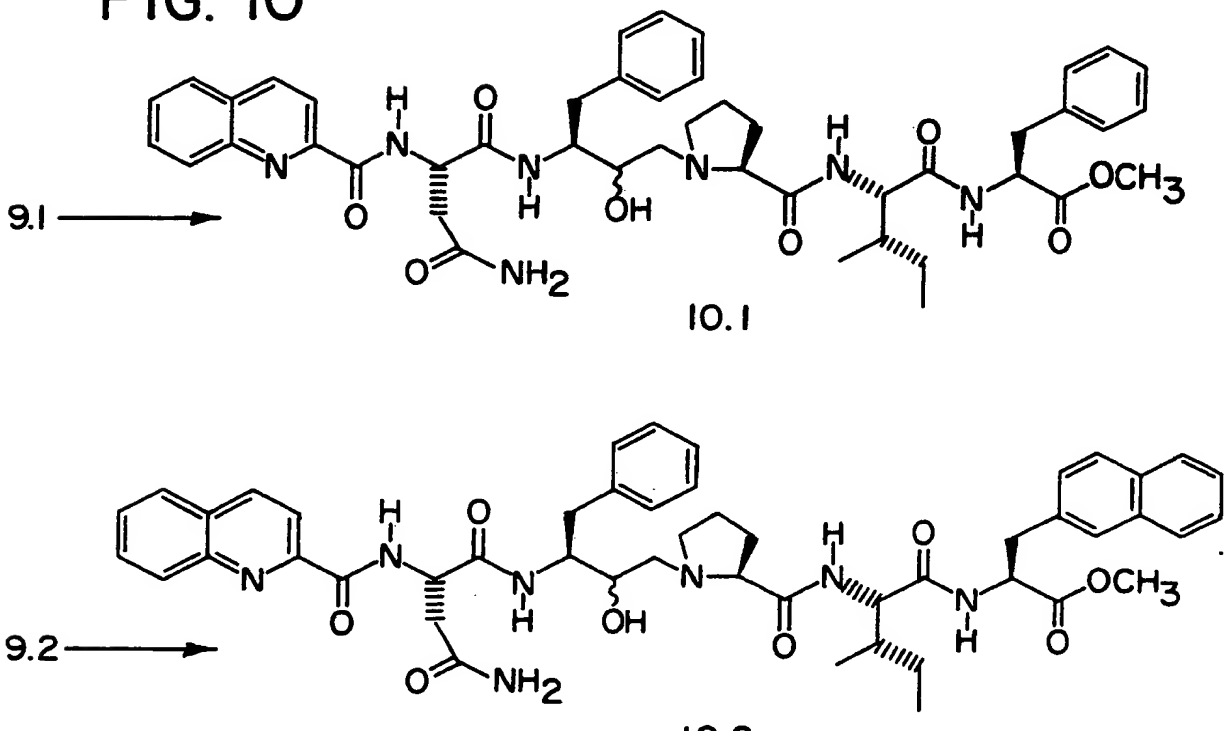

7.1 + 8.2
Z-HN
OCH3
NH2
OH
9.1
7.1 + 8.4
Z-HN
OCH3
NH2
OH
9.2

FIG. 10


9.1
OCH3
NH2
OH
10.1
9.2
OCH3
NH2
OH
10.2

M·H

# PATENT COOPERATION TREATY

# PCT

REC'D 15 FEB 2000

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

**(PCT Article 36 and Rule 70)**

| Applicant's or agent's file reference 609.1PC | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No. PCT/US98/25964 | International filing date *(day/month/year)* 08 DECEMBER 1998 | Priority date *(day/month/year)* 08 DECEMBER 1997 |
| International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet. | | |
| Applicant THE SCRIPPS RESEARCH INSTITUTE | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 15 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☒ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 07 JULY 1999 | 20 JANUARY 2000 |
| Name and mailing address of the IPEA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231 | Authorized officer<br>JEFFREY E. RUSSEL |
| Facsimil No. (703) 305-3230 | Telephone No. (703) 308-0196 |

Form PCT/IPEA/409 (cover sheet) (January 1994)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/25964

## I. Basis of the report

1. This report has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments)*:

[X] the international application as originally filed.

[X] the description, pages 1-68 , as originally filed.
pages NONE , filed with the demand.
pages NONE , filed with the letter of _____.
pages _____ , filed with the letter of _____.

[X] the claims, Nos. 1-5 , as originally filed.
Nos. NONE , as amended under Article 19.
Nos. NONE , filed with the demand.
Nos. NONE , filed with the letter of _____.
Nos. _____ , filed with the letter of _____.

[X] the drawings, sheets/~~fig~~ 1-16 , as originally filed.
sheets/~~fig~~ NONE , filed with the demand.
sheets/~~fig~~ NONE , filed with the letter of _____.
sheets/~~fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[X] the description, pages NONE.

[X] the claims, Nos. NONE.

[X] the drawings, sheets/~~fig~~ NONE.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/25964

**V. Reasoned statement under Article 35(2) with regard t nov lty, inv ntiv step r industrial applicability; citati ns and xplanati ns supp rting such stat ment**

1. STATEMENT

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1 | YES |
| | Claims | 2-5 | NO |
| Inventive Step (IS) | Claims | NONE | YES |
| | Claims | 1-5 | NO |
| Industrial Applicability (IA) | Claims | 1-5 | YES |
| | Claims | NONE | NO |

2. CITATIONS AND EXPLANATIONS

Claim 1 lacks an inventive step under PCT Article 33(3) as being obvious over Japan Energy Corporation Tokyo-To. Japan Energy Corporation Tokyo-To teaches an HIV-protease inhibitor at column 3, lines 25-32, and page 10, lines 1-19, which differs from Applicant's claimed protease inhibitors in that Japan Energy Corporation Tokyo-To, for the compound of page 10, does not specifically teach X being methylene, $R_{21}$ and $R_{22}$ being H, and $R_3$ being t-butyl, although these possibilities are embraced by the generic formula at page 3. It would have been obvious to one of ordinary skill in the art at the time Applicant's invention was made to form a compound according to Japan Energy Corporation Tokyo-To in which X is methylene, $R_{21}$ and $R_{22}$ are H, and $R_3$ is t-butyl, because these possibilities are embraced by the generic formula, because the choice of X being methylene and $R_{21}$ and $R_{22}$ being H results in the presence of a conventional proline residue, because the choice of $R_3$ being t-butyl is the choice of homologous small alkyl groups, and because the resulting compound has only the expected protease inhibitory activity.

Claim 2 lacks novelty under PCT Article 33(2) as being anticipated by Abbott Laboratories. Abbott Laboratories at claims 1 and 2 teaches a protease inhibitor which has the same structure as the compound recited in Applicant's claim 2.

Claim 2 lacks an inventive step under PCT Article 33(3) as being obvious over Baker et al. Baker et al teach an HIV-protease inhibitor at column 1, lines 53-64 and column 2, lines 1-17, which differs from Applicant's claimed protease inhibitors in that Baker et al, for the compound of column 2, does not specifically teach n=0, $R_2$ and $R_{2a}$ being carbobenzoxy, and $R_1$ being the sidechain for valine, although these possibilities are embraced by the generic formula at claim 1. It would have been obvious to one of ordinary skill in the art at the time Applicant's invention was made to form a compound according to Baker et al in which n=0, $R_2$ and $R_{2a}$ are carbobenzoxy, and $R_1$ is the sidechain for valine, because these possibilities are embraced by the generic formula, because n=0 is one of only two possibilities for n and is the possibility present in the more (Continued on Supplemental Sheet.)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/25964

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 1 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim is indefinite for the following reason(s): At claim 1, line 23, the alternative to the phrase "are either a single combined oxygen forming a carbonyl group" is not recited.

**Supplemental Box**
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII



CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(6): A61K 31/045, 31/13, 31/135, 31/165, 31/38, 31/405, 31/47, 37/05; C07C 205/02, 211/09, 215/18; C07D 207/16, 217/16, 217/26, 277/24, 277/28; C07K 5/062, 5/065 and US Cl.: 514/18, 19, 307, 365, 423, 483, 487, 488, 489, 654, 667, 669; 530/330; 546/146; 548/204, 537; 560/21, 22, 156; 564/374, 381, 503, 507

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
preferred embodiment at column 2, formula 3, because carbobenzoxy is a conventional N-blocking group, because the choice of $R_1$ being the sidechain for valine is the choice for homologous small alkyl groups and is the possibility present in the more preferred embodiment at column 2, formula 3, and because the resulting compound has only the expected protease inhibitory activity.

Claim 2 lacks novelty under PCT Article 33(2) as being anticipated by Dreyer et al. Dreyer et al at page 941, Table II, teaches protease inhibitors which have the same structure as the compound recited in Applicant's claim 2.

Claim 2 lacks an inventive step under PCT Article 33(3) as being obvious over Kempf et al. Kempf et al teach an HIV-protease inhibitor at column 2, line 39 - column 6, line 50, and at claim 4 which differs from Applicant's claimed protease inhibitors in that Kempf et al, for the compound of claim 4, has a heterocyclic group rather than a phenyl group at $R_7$, although this possibility is embraced by the generic formula at column 4, line 67 - column 5, line 4. It would have been obvious to one of ordinary skill in the art at the time Applicant's invention was made to form a compound according to Kempf et al in which in the compound of claim 4, $R_7$ is phenyl, because this possibility is embraced by the generic formula, because carbobenzoxy is a conventional N-blocking group, and because the resulting compound has only the expected protease inhibitory activity.

Claim 3 lacks novelty under PCT Article 33(2) as being anticipated by Handa et al. Handa et al at Examples 15 and 85 teaches compounds which have the same structure as the compound recited in Applicant's claim 3.

Claim 4 lacks novelty under PCT Article 33(2) as being anticipated by Thompson et al. Thompson et al at column 2, lines 31-48, and column 7, lines 26-39 teaches protease inhibitors which have the same structure as the compound recited in Applicant's claim 4.

Claim 5 lacks novelty under PCT Article 33(2) as being anticipated by Tien et al. Tien et al at column 4, lines 1-15, teaches a protease inhibitor which has the same structure as the compound recited in Applicant's claim 5.

Claims 1-5 meet the criteria set out in PCT Article 33(4). The claimed invention would have been expected to have industrial applicability in the inhibition of protease.

---------- NEW CITATIONS ------ ----------
NONE

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
**A61K 31/045, 31/13, 31/135, 31/165, 31/38, 31/405, 31/47, 38/05, C07C 205/02, 211/09, 215/18, C07D 207/16, 217/16, 217/26, 277/24, 277/28, C07K 5/062, 5/065**

**A1**

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(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/067,959 (CIP)
Filed on 8 December 1997 (08.12.97)

(71) Applicant *(for all designated States except US)*: THE SCRIPPS RESEARCH INSTITUTE [US/US]; 10550 North Torrey Pines Road, La Jolla, CA 92037 (US).

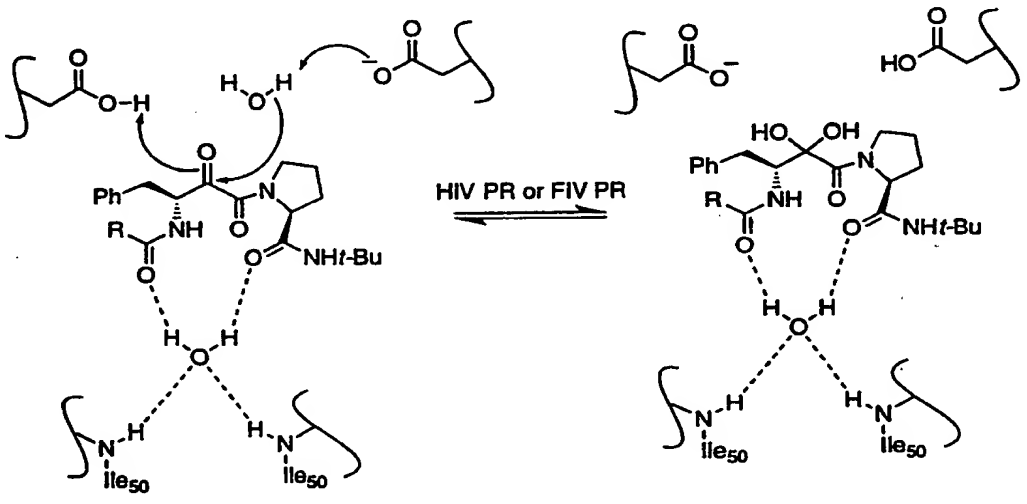
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LEE, Taekyu [CA/US]; 5510 Limeric Circle, Apt. 44, Wilmington, DE 19808 (US). WONG, Chi-Huey [US/US]; P.O. Box 8154, Rancho Santa Fe, CA 92067 (US). ELDER, John, H. [US/US]; P.O. Box 7002, Rancho Santa Fe, CA 92067 (US).

(74) Agents: LEWIS, Donald, G. et al.; The Scripps Research Institute, 10550 North Torrey Pines Road, TPC-8, La Jolla, CA 92037 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*

(54) Title: HIV/FIV PROTEASE INHIBITORS HAVING A SMALL P3 RESIDUE




(57) Abstract

With the help of X-ray structural analyses of drug-resistant HIV proteases and molecular modeling, a new type of inhibitor with a small P3 residue has been developed. These inhibitors are effective against HIV and its drug-resistant mutants, as well as FIV. Modification of existing HIV protease inhibitors by reducing the size of the P3 residue has the same effect. This finding provides a new strategy for the development of HIV protease inhibitors effective against the wild type and drug-resistant mutants and further supports that FIV protease is a useful model for drug-resistant HIV proteases, which often are developed through reduction in size of the binding region for the P3 group or the combined P3 and P1 groups.

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25964

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 0 751 145 A2 (JAPAN ENERGY CORPORATION TOKYO-TO) 02 January 1997 (02/01/97), page 3, lines 25-32, page 10, lines 1-19. | 1 |
| X | WO 93/23361 A1 (ABBOTT LABORATORIES) 25 November 1993 (25/11/93), claims 1 and 2. | 2 |
| X | US 5,541,321 A (BAKER ET AL) 30 July 1996 (30/07/96), column 1, lines 53-64, column 2, lines 1-17. | 2 |
| X | DREYER et al. A Symmetric Inhibitor Binds HIV-1 Protease Asymmetrically. Biochemistry. 1993, Volume 32, Number 3, pages 937-947, especially page 941, Table II. | 2 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
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| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
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| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>09 FEBRUARY 1999 | Date of mailing of the international search report<br>25 FEB 1999 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>JEFFREY E. RUSSEL<br>Telephone No. (703) 308-0196 |

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25964

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5,354,866 A (KEMPF ET AL) 11 October 1994 (11/10/94), column 2, line 39 - column 6, line 50, column 6, line 67 - column 7, line 3. | 2 |
| X | US 5,157,041 A (HANDA ET AL) 20 October 1992 (20/10/92), abstract, Examples 15, 85. | 3 |
| A | US 5,552,405 A (GORYS ET AL) 03 September 1996 (03/09/96), abstract. | 3 |
| X,P | US 5,733,906 A (JUNGHEIM ET AL) 31 March 1998 (31/03/98), column 2, line 17 - column 3, line 28, column 9, lines 17-40. | 3, 4 |
| X | US 5,502,060 A (THOMPSON ET AL) 26 March 1996 (26/03/96), column 2, lines 31-48, column 7, lines 26-39. | 4 |
| X | US 5,567,823 A (TIEN ET AL) 22 October 1996 (22/10/96), column 4, lines 1-15. | 5 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25964

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/045, 31/13, 31/135, 31/165, 31/38, 31/405, 31/47, 38/05; C07C 205/02, 211/09, 215/18; C07D 207/16, 217/16, 217/26, 277/24, 277/28; C07K 5/062, 5/065

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/18, 19, 307, 365, 423, 483, 487, 488, 489, 654, 667, 669; 530/330; 546/146; 548/204, 537; 560/21, 22, 156; 564/374, 381, 503, 507

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

514/18, 19, 307, 365, 423, 483, 487, 488, 489, 654, 667, 669; 530/330, 331; 546/146; 548/204, 537; 560/21, 22, 156; 564/374, 381, 503, 507

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CHEMICAL ABSTRACTS
search terms: hiv, fiv, protease, proteinase, inhibit, carbobenzoxy, thiazol, proline, isoquinoline, structures of claims 1-5